ON THE LUMINESCENCE OF *MICROSCOLEX PHOSPHOREUS* DUG.[1]

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In spite of several papers describing observations on the luminescence of different species of earthworms, there are still some uncertain points which deserve further investigation. In Mangold's ('14) article, which discusses thoroughly the power of luminescence in all groups of plants and animals, the conclusions lead to the assumption that the production of light in these animals is due to infection by luminous microorganisms or fungi, which may be present in places where earthworms obtain their food. Dubois ('14) and Linsbauer ('17) were unable, however, to show in the luminous slime any luminous microörganisms, which is a strong argument against the view of the bacterial origin of luminescence in this group of the animal kingdom. Gilchrist ('19) working on the South African earthworm species *Chilota*, describes many observations and experiments which seem to show that the luminescence in this species is not bacterial. Quite recently, however, Pierantoni ('24), according to his "hereditary symbiosis theory," believes that *Microscolex phosphoreus* owes its luminescence to symbiotic bacteria living inside the cells of its body. It is the purpose of this paper to show how the luminescence in *Microscolex* originates, and which opinion rests upon a sounder basis.

Observations and Experiments.

Microscolex phosphoreus,[2] a small earthworm with clitellum situated near the anterior part of the body is easily to be found in Naples and its environs. If the animal be kept undisturbed, one cannot observe the production of luminous slime, which is

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discharged only on mechanical, chemical or electrical stimulation. It may be mentioned here that all the specimens of *Microscolex phosphoreus* examined by me were found to be luminescent. In free living animals the small hard particles of the soil may produce, by irritating the skin, the necessary stimulus for the secretion of the slime, which glows brightly with a yellow-greenish tinge. The slime is usually ejaculated from the anal aperture, and only in some specimens kept previously for a longer time undisturbed, did the slime flow also from the mouth opening. The posterior and the anterior end of the alimentary canal were the only two points at which the production of luminous slime was observed. As all these observations were carried out under the binocular microscope it is quite certain that the glands of the skin and the excretory system do not take part in the secretion of luminous slime. If the animal be wounded, however, on any point of the body, the luminescent slime flows from this part, the clitellum not being an exception. Nevertheless the luminous material does not arise from the alimentary canal, because in such cases the luminescence appears when the wound has not reached the intestinum. An injury of the body cavity is sufficient in itself for a production of luminous slime, which does originate, therefore, from the cœlomic fluid. This fact was corroborated by microscopical observations. A freshly discharged slime is composed chiefly of round cells with many, strongly light-refracting granules, scattered inside the protoplasm. Cells of this kind are found abundantly in the body cavity. Besides the uninjured cells, a certain amount of free granules, liberated by breaking of the cells, is always suspended in the fluid. I wish to call attention to the fact that both the size of those cells, and the structure of the intestinum, excludes the possibility of any other way of reaching the alimentary canal by the cells, except by preformed openings at its posterior and anterior end. That the communication with the body cavity is possible in *Oligochæta* was also shown by Gilchrist in *Chilota*, though in *Microscolex* the posterior rather than the anterior part of the intestine is the chief point at which ejaculation of the luminous slime occurs. Benham ('99) found that in the New Zealand earthworm, *Octochætus multiporus*, the cells of the luminous slime take their origin from the body cavity. The fluid is discharged, however, "from the dorsal pores and from the mouth, which it reaches through the peptonephridia opening into the buccal cavity." These observations show clearly that it is most improbable that the granules alone (Pierantoni's bacteria) may pass through the connective tissue and two layers of muscular system until they reach the glands of the skin as Pierantoni seems to believe.

The next task was to find out whether the cells or the granules alone have the power of luminosity. To test this the luminous extract, obtained by grinding up the whole animals, which easily passes through ordinary filter paper without loosing its luminescence, was centrifuged for 15 min. The centrifuged fluid in which only granules were seen could be made luminous on addition of a few drops of ether. After filtration through bacteriological filters, however, no light was observed upon treatment with ether. The granules therefore must be regarded as a luminous material in *Microscolex*. This agrees with Gilchrist's observations on *Chilota*.

It was noticed that the power of the ejaculation of the slime is directly proportional to the degree of the irritability of the nervous system. Luminous microörganisms, however, glow continually, and are not affected by nerve stimulation when living in luminous organs of some other forms (in certain fishes and cephalopods). A lowered temperature also affects the luminescence, probably by the lessened irritability which is especially noticeable if the animals be kept for a long time at 8°–10° C. In connection with this I should like to mention the day-night rhythm of luminescence in *Microscolex*. It was observed that if stimulated, the animal discharged more easily the luminescent slime during the night than in the daytime. It can be shown that this rhythm does not result from an inhibition by light, because the strong arc lamp and sunlight have no visible effect on luminescence. It is possible that this rhythm may be explained by the general lower irritability of the animals during the daytime.

Before the characteristics of the luminescence will be discussed, it must be stated that if the properties of the luminous slime be compared with those of an extract from the ground worms, it is easy to find a very striking difference. The slime continues to glow for about half an hour if kept moist, while on the contrary the light of the extract dies quickly, and on addition of water, immediately. Filter paper saturated with slime gives off light after an hour and a half if moistened; the dried extract, however, prepared from the ground worms, does not show any luminescence. It is difficult to explain these facts by oxydation of luminous material alone, especially as the experiments of Gilchrist show that pure luminous slime of *Chilota* mixed with water glows brightly for over two hours. Acidity does not seem to play an important rôle in the above mentioned observations, as solutions with different pH did not prolong the luminescence. It seems more probable that this difference may be due to some enzymes liberated from the cells by grinding, which have an inhibiting effect upon the luminous material.

If to luminous bacteria some chloroform or ether be added, the light becomes fainter and dies out quickly. On the contrary the extract from certain luminous animals such as *Pelagia* gives a bright flash of light on addition of fresh or distilled water, ether, saponin, etc. (E. N. Harvey, A. R. Moore). The extract of *Microscolex* reacts like that of *Pelagia*. Of the cytolytic substances tried—ether, chloroform, saponin, sodium glycocholate—only ether gives positive results. The light was however never as bright as in the living animal, but still visible. Owing to the rather small size of the animals it was difficult to experiment with pure luminous slime, but a few observations showed that in this case the action of the cytolytic substances were more clearly seen. Ether and chloroform produce the brightest luminescence of luminous slime of *Microscolex* and the same effect was observed by rubbing it with a finger.

E. N. Harvey supposes that the action of cytolytic substances may cause the cyto- or granulolysis of luminous bodies. In *Microscolex* it was difficult to observe directly the changes in the granules themselves, but after a longer time the slime treated previously with ether shows swelling of the luminous granules.

It may be remarked that NaF in 1.5 per cent. concentration has no inhibiting effect on luminescence of the slime or the extract of *Microscolex*. The light of free living luminous bacteria, however, ceases quickly in this solution and the same was observed by E. N. Harvey on the symbiontes multiplying in the luminous organs of the two fishes, *Photoblepharon* and *Anomalops*, and by me in *Sepiola intermedia*.

The presence of oxygen is necessary for the luminescence of *Microscolex*. It is obvious that the behavior of the earthworms does not differ on this point from many luminous organisms such as *Cypridina*, firefly, bacteria and others. In an atmosphere of hydrogen the luminescence disappears quickly and does not return on mechanical or electrical stimulation. After removing the animals from hydrogen the luminescence appears again. The luminous slime kept on a slide or the filter paper impregnated with slime glows brightly upon moistening with water even after an hour and a half. After this time no light can be observed. Under the cover-glass however, if the edges be covered with vaseline, the light appears after many days in the presence of oxygen and water. This observation confirms fully the data collected by Gilchrist in *Chilota* and shows that the disappearance of luminescence points to the complete oxidation of the luminous material.

If the ground animals be mixed with water to which some sodium hyposulphite has been added to remove the oxygen, and a few drops of methylene blue which serves as an indicator for the presence of oxygen, the light which appears on addition of ether is visible after a longer time, and is brighter than in the control tube without the sodium hyposulphite. The flash of light is observed at the moment when on shaking, the oxygen dissolves in sufficient quantity so as to change the colorless methylene blue to the colored compound. This is apparently due to the nonoxidation of some of the granules, which were not completely destroyed by the action of enzymes or perhaps to the lessened activity of these enzymes due to the modified composition of the solution.

The different tissues of *Microscolex* contain a sufficient quantity of oxygen to allow luminescence as proved by vital staining with methylene blue. Nevertheless the light is not produced continually as in luminous bacteria, which fact must be taken as contradictory to the bacterial origin of luminescence in this animal.

It is well known that luminous bacteria cease to glow at a lower temperature than the secretions of animals which have luminescence of their own. Furthermore one of the constant characteristics of the bacterial light is the reappearance of the luminescence after cooling and of the same intensity as before heating. The living *Microscolex* kept in fresh water does not give off any light upon addition of ether or alcohol if previously heated to 60° C. The same was noticed if the dried material or the filter paper impregnated with luminous slime were tested for luminescence by pouring on them water heated to different temperatures from 55°–60° C. It may be mentioned here that after cooling the luminescence did not return in any case. These observations agree with the results obtained in other luminous animals whose self-luminescence is beyond any doubt, and differ widely from the behavior of luminous bacteria. Different results were obtained, however, with solutions prepared from the whole worms. In this case the maximum of temperature at which the light was observed reaches but 40° C. This difference may be explained by the quicker destruction of the luminous granules by the substances (enzymes) liberated from the rest of the body. Should this explanation prove true one cannot expect to find in the solution at a higher temperature unaltered luminous material which may react with the added ether.

In the older literature one finds statements that the dried luminous slime of the earthworms can be made luminous again by application of water. The same was found in my observations and this experiment can be repeated many times until complete oxidation of the luminous material. If the whole animals be dried in an evacuated desiccator the ground powder behaves like the fresh discharged slime, the intensity of luminescence being about the same. Even after three months the dry powder glows brilliantly if in water, the luminescence lasting for some time relatively to the amount of the powder. The dried bacteria however can be made luminous by moisture but only for a shorter time after desiccation, and the light is much fainter than that of the living microörganisms.

Pierantoni states that the addition of sodium chloride to the water in which the living specimens of *Microscolex phosphoreus* are kept inhibits their luminescence. In spite of many trials I have not been able to demonstrate the effect of different salt solutions when added to the powder from desiccated earthworms. The observations on *Microscolex* agree therefore with those of E. N. Harvey on *Cypridina* where the effect of different ions was not to be found. Pierantoni in his paper distinguishes two types of luminescence in *Microscolex*. Besides the external secretion of luminous slime he describes a general luminescence of the whole body, first visible at the posterior and anterior part, which takes place within the different tissues. This internal luminescence may be produced by the strong stimulating action of ammonia or alcohol. It seems to me that in many cases the ejaculated slime, through the movements of the animal, spreads over the whole body and sticks to the skin, thus imitating an internal luminescence. By rubbing the worm over the filter paper or cloth all the slime can be removed but in water, however, the viscous slime does not dissolve easily. In ammonia of the proper strength the motionless animal begins to glow, the posterior part glowing first. After some time the luminosity spreads over the whole body even if the animal be apparently dying. The same effect can be obtained with alcohol solution. With ether or chloroform I noticed only the external luminescence, the movements of the worm being for a longer time very energetic. Ammonia arrests almost immediately the muscular action. It is therefore to be understood that only in a few cases the ejaculation of the slime on application of ammonia was observed. The luminosity of the dying animal may be compared with the steady death glow as noticed in *Noctiluca* (E. B. Harvey, '17) and other forms. This internal luminescence may be hastened if the worm be rubbed gently with the finger when in ammonia or alcohol. If the animals are in a very poor condition they can also be made luminous by rubbing them, even without ammonia or alcohol. Sometimes the worms already luminescent can, if slightly pressed, throw the luminous slime through the anal or oral aperture. After the ejaculation of slime the animal loses its luminosity, which indicates that the internal luminescence cannot be taken as a different type from the external one. Pierantoni's view, which explains the internal luminosity as the continual glowing of the symbiontes inside the cells of the different tissues of the body, seems to me, therefore, a very improbable hypothesis.

Discussion.

Summing up all the characteristics of the luminescence of *Microscolex phosphoreus* (the rôle of the nervous system, the effect of ether, temperature, the luminosity of desiccated worms) we can see that they do not agree with the observations on the properties of bacterial light but point to a luminescence of its own in this species of *Oligochæta*. It is very improbable that the luminous symbiontes may change their type of luminescence so completely as to behave like such animals as *Cypridina*, *Pholas*, *Noctiluca* and others, especially as the luminous bacteria living in luminous organs in some fishes and Cephalopods do not show such differences, though their shape may be modified. *Microscolex* and *Chilota* are therefore at least two earthworm species which are certainly not infected with luminous bacteria. *Allolobophora fœtida* (Vejdowsky) however may be occasionally infected by microörganisms or fungi, as only a few luminous specimens were found.

As I have mentioned before, the luminous material in *Microscolex* consists of small granules which usually begin to glow after the slime has been discharged. If the animal be dying however, they become luminous within the body cavity. It may be thought that the luminescence of the granules depends on an amount of oxygen which is not present in sufficient quantity in cœlomic fluid. If the worm be dying the amount of oxygen may be increased, but the experiments carried out by E. N. Harvey did not show any difference in the rate of permeability for oxygen between living and dead cells. One may suppose however that the luminescence begins when the cells disintegrate, which is corroborated by the fact that on rubbing the brightness of the slime increases. The process of breaking up the cells with granules within was observed by me several times after the slime was discharged. On one occasion Gilchrist noticed that the light was given off from the glowing particles situated in cells, which were in process of breaking up. I do not think that the granules can be luminous only during the time when they begin to be thrown off from the cells, for the centrifuged extract containing the free granules alone gives off light if ether be added, but it may be that they become luminous after they are liberated. It is possible that if the worm be dying the cells break up inside the body cavity. This process never occurs if the animal is in good condition. The accelerating effect of the rubbing may be explained by the increase of the process of breaking up the cells. In favor of such a view it may be noticed that the ejaculated slime which glows brightly always contains free granules in abundance, and their amount increases with time when the cells dissolve. It was impossible to prove this directly as the light of the granules is not strong enough to be seen under the microscope.

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Summary.

Microscolex phosphoreus is characterized by an external luminescence (except the steady death glow) which begins upon stimulation. All the properties of its light seem to show that this species has a luminescence of its own. The luminous material is represented by small granules situated in the protoplasm of the cells, which take their origin from the body cavity. The luminescence begins probably after the granules are liberated from the cells.

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